

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/27182>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

ROLE OF LFA-1 AND VLA-4 IN THE ADHESION OF CLONED NORMAL AND LFA-1 (CD11/CD18)-DEFICIENT T CELLS TO CULTURED ENDOTHELIAL CELLS

Indication for a New Adhesion Pathway

CLAUS J. G. M. VENNEGOOR,^{1*} ELLY VAN DE WIEL-VAN KEMENADE,^{2*}
RICHARD J. F. HUIJBENS,* FRANCISCO SANCHEZ-MADRID,[†] CORNELIS J. M. MELIEF,^{3*}
AND CARL G. FIGDOR^{4*}

From the *Division of Immunology, The Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; and the [†]Service Immunologia, Hospital de la Princesa, Diego de León 62, 28006 Madrid, Spain

Patients with the leukocyte adhesion deficiency (LAD) syndrome have a genetic defect in the common β_2 -chain (CD18) of the leukocyte integrins. This defect can result in the absence of cell surface expression of all three members of the leukocyte integrins. We investigated the capacity of T cell clones obtained from the blood of an LAD patient and of normal T cell clones to adhere to human umbilical vein endothelial cells (EC). Adhesion of the number of LAD T cells to unstimulated EC was approximately half of that of leukocyte function-associated antigen (LFA)-1⁺ T cells. Stimulation of EC with human rTNF- α resulted in an average 2- and 2.5-fold increase in adhesion of LFA-1⁺ and LFA-1⁻ cells, respectively. This effect was maximal after 24 h and lasted for 48 to 72 h. The involvement of surface structures known to participate in cell adhesion (integrins, CD44) was tested by blocking studies with mAb directed against these structures. Adhesion of LFA-1⁺ T cells to unstimulated EC was inhibited (average inhibition of 58%) with mAb to CD11a or CD18. Considerably less inhibition of adhesion occurred with mAb to CD11a or CD18 (average inhibition, 20%) when LFA-1⁺ T cells were incubated with rTNF- α -stimulated EC. The adhesion of LFA-1⁻ T cells to EC stimulated with rTNF- α , but not to unstimulated EC, was inhibited (average inhibition, 56%) by incubation with a mAb directed to very late antigen (VLA)-4 (CDw49d). In contrast to LAD T cell clones and the LFA-1⁺ T cell line Jurkat, mAb to VLA-4 did not inhibit adhesion of normal LFA-1⁺ T cell clones to EC, whether or not the EC had been stimulated with rTNF- α . We conclude that the adhesion molecule pair LFA-1/intercellular adhesion molecule (ICAM)-1 plays a major role in the adhesion

of LFA-1⁺ T cell clones derived from normal individuals to unstimulated EC. Adhesion of LFA-1⁻ T cells to TNF- α -stimulated EC is mediated by VLA-4/vascular cell adhesion molecule (VCAM)-1 interactions. Since we were unable to reduce significantly the adhesion of cultured normal LFA-1⁺ T cells to 24 h with TNF- α -stimulated endothelium with antibodies that block LFA-1/ICAM-1 or VLA-4/VCAM-1 interactions, and lectin adhesion molecule-1 and endothelial leukocyte adhesion molecule-1 appeared not to be implicated, other as yet undefined cell surface structures are likely to participate in T cell/EC interactions.

Lymphocyte traffic allows continuous immune surveillance of the body. In secondary lymphoid organs including lymph nodes, tonsils, adenoids, Peyer's patches, appendix, and mucosa-associated lymphoid tissues leukocyte extravasation occurs predominantly in specialized postcapillary venules which are lined by high endothelium (see Reference 1 for review).

Some of the adhesive properties of high endothelium in inflamed tissue can be mimicked by exposing cultured human umbilical vein EC⁵ to cytokines such as TNF- α (2, 3), IL-1 (2, 3), or LPS (2). In man, leukocyte/EC interactions may therefore be studied with cytokine-treated allogeneic cultured umbilical vein EC.

Several sets of adhesion molecules, e.g., the ligand/receptor pairs leukocyte function-associated antigen (LFA)-1 intercellular adhesion molecule (ICAM)-1 (4), LFA-1/ICAM-2 (5), very late antigen (VLA)-4/vascular cell adhesion molecule (VCAM)-1 (6, 7), the lymphocyte homing receptor lectin adhesion molecule (LECAM)-1 (8, 9), endothelial leukocyte adhesion molecule (ELAM)-1 (10, 11), and the endothelial granule membrane protein-140 molecule (12) are involved in the antigen-independent interaction of EC and leukocytes. The human counterstructures of the last three molecules are as yet partly characterized (13). LFA-1 integrin expression on T cells from normal individuals strongly contributes to binding of these cells to their counterstructures on EC (11, 14,

Received for publication August 23, 1991.

Accepted for publication November 22, 1991.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Current address: Clinical Support Laboratory, Department of Gynaecology and Obstetrics, Academical Hospital of the Free University of Amsterdam, de Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

² Supported by Dutch Cancer Society Grant NKI 87-5.

³ Current address: Department of Immunohaematology, Academical Hospital Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

⁴ Address correspondence and reprint requests to Carl G. Figdor, Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

⁵ Abbreviations used in this paper: EC, endothelial cell; PBS-BSA, PBS containing 0.01% CaCl₂, 0.01% MgCl₂ and 0.25% BSA; LFA, leukocyte function-associated antigen; ICAM, intercellular adhesion molecule; VCAM-1, vascular cell adhesion molecule-1; LECAM-1, lectin adhesion molecule-1; ELAM-1, endothelial leukocyte adhesion molecule-1; VLA, very late antigen; LAD, leukocyte adhesion deficiency; HEV, high endothelial venule; ECGF, endothelial cell growth factor.

15), thereby veiling the possible involvement of other molecules in the leukocyte/EC interaction. Therefore, in this study we investigated the adhesive properties of LFA-1⁻ T cells derived from a patient with leukocyte adhesion deficiency (LAD) to determine the contribution of other cell surface molecules in T cell/EC interactions. Complexes of α' and β' precursors of leukocyte integrins are required for processing and transport to the cell surface (16). We generated T cell clones from peripheral blood of a patient with LAD whose leukocytes completely lack cell surface expression of the three leukocyte integrin molecules LFA-1, Mac-1, and p150,95 as a result of the incapability of the cells to produce mRNA coding for the β_2 subunit of the leukocyte integrins.⁶ Such patients suffer from life-threatening recurrent bacterial infections and persistent neutrophilia. The impaired granulocyte-monocyte function caused by this defect is demonstrated by the failure of migration and adhesion of the polymorphonuclear leukocytes. LAD patients, however, have shown no clinical manifestation of defects in T cell or NK cell functions, suggesting that other adhesion pathways could be effectively utilized in lymphocytes *in vivo* to compensate for CD11/CD18 deficiency in these cells (17).

In this study we have characterized cell surface molecules that are involved in the adhesion of cytotoxic and noncytotoxic CD4⁺ and cytotoxic CD8⁺ T cell clones from both normal individuals and an LAD patient. The results demonstrate that the LFA-1/ICAM-1 interaction predominantly mediates binding of T cells from healthy individuals to unstimulated endothelium and that VLA-4/VCAM-1 interaction plays a major role in the binding of T cells from LFA-1⁻ patients to EC activated for 24 h by TNF- α .

MATERIALS AND METHODS

EC. Human EC were isolated from umbilical vein by collagenase digestion (18). The cells were cultured in 0.2% gelatin-coated 75-cm² flasks (3275, Costar, Cambridge, MA) in M199 (Flow Laboratories, Irvine, Scotland) in the presence of penicillin and streptomycin (GIBCO Europe, Paisley, Scotland), 10% heat-inactivated human serum (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands), bovine hypothalamus ECGF (5 μ g/ml, Boehringer Mannheim, FRG), heparin (10 μ g/ml, Sigma, St. Louis, MO), BSA (0.25%, fraction V, Boehringer Mannheim), and the ingredients transferrin (0.036%), bovine pancreas insulin (5 μ g/ml), linoleic acid (1 μ g/ml), oleic acid (1 μ g/ml), and palmitic acid (1 μ g/ml, all from Sigma), which have been described as components of a chemically defined medium for generating T cell clones (19). The cells were serially passaged by trypsin treatment. Cells from passages 4 through 7 were used for the experiments. More than 95% of the cells were considered EC as they stained with mAb CLB-RAG-35 to the von Willebrand factor (20) in an immunofluorescence test on acetone-fixed cells grown on fibronectin-coated coverslips.

T cells. The allo-MHC specific cytotoxic T cell clones JS132 (CD8⁺) and JS136 (CD4⁺) were from the same individual and have been described previously by Borst et al. (21). The tetanus toxoid specific T-helper clone HY827 has been described by Yssel et al. (22). The LFA-1-negative T cell clones LAD1, LAD4, LAD6.6, and LAD19 were raised by limiting dilution of PBL from a patient with LAD lacking mRNA expression for the CD18 protein.⁶ The clones were grown in Iscove's medium (Flow Laboratories) containing 5% heat-inactivated human serum by weekly stimulation with 2 \times 10⁵ irradiated allogeneic PBL/ml and 1 \times 10⁵ EBV-transformed B cell line JY cells/ml in the presence of PHA (0.2 μ g/ml) and IL-2 (100 Cetus U/ml; 3.2 \times 10⁶ Cetus U/mg; Cetus Corp., Emeryville, CA). To avoid interference in the adhesion assays, the T cells were maintained in the presence of IL-2 but in the absence of feeder cells and PHA for 1 wk before testing. The EBV-transformed B cell line JY and the T cell line Jurkat were cultured in Iscove's medium containing 10% FCS.

⁶ Van de Wiel-Van Kemenade, E., A. A. Te Velde, A. J. De Boer, R. S. Weening, C. J. M. Melief, and C. G. Figdor. Cytotoxic activity of LFA-1 negative T cells is mediated through CD2/LFA-3. *Submitted for publication.*

Monoclonal antibodies. The mAb used in the adhesion test and their final concentrations are listed in Table I. No differences were observed between HPLC-purified antibodies and ascites. Additional mAb used for phenotyping T cell clones and EC were obtained from several laboratories: CLB-10G11 (anti-VLA-2, CDw49b) (44) and C17 (anti- β_3 -integrin, CD41) (45) from A. Sonnenberg, Amsterdam; CLB-IL2R/1 (anti-IL-2R, CD25) (46) from R. van Lier, Amsterdam; CLB-FcR-gran1 (anti-Fc receptor III, CD16) (47) from P. Tetteroo, Amsterdam; CLB-RAG-35 (anti-von Willebrand factor) (20) from J. van Mourik, Amsterdam; ENA-2 (anti-ELAM-1)⁷ from J. Leeuwenberg, Maastricht; J143 (anti-VLA-3, CDw49c) (48) from E. Klein, Ulm; B-ly 6 (anti-p150,95, CD11c) (49) from S. Poppema, Edmonton; TS2/16 (anti- β_1 -integrin, CD29) (50) from T. Springer, Boston; and B-5G10 (anti-VLA-4, CDw49d) (51) from M. Hemler, Boston. Leu8 (anti-LECAM-1) was obtained from Becton and Dickinson, San Jose, CA. 32.2 (anti-Fc receptor I, CD64) (52) and IV.3 (anti-Fc receptor II, CD32) were purchased from Medarex Inc., West Lebanon, NH. NKI-Bear1 (anti-CR3, CD11b) (27) and HP1/3 (anti-VLA-4, CDw49d) (37) have been described previously.

Adhesion test. Endothelial cells were trypsin treated, and 2 \times 10⁴ cells in 100 μ l of M199 containing 20% heat-inactivated human serum (but without ECGF, heparin, and the components of chemically defined medium; see above) were seeded into 0.2% gelatin-coated 96-well plates (3596, Costar). After incubation overnight the cells were stimulated with human rTNF- α (supernatant of cDNA-transfected COS cells), using the concentrations indicated in the experiments. Before initiation of the adhesion experiment the wells were washed twice with 0.3 ml of sterile PBS-BSA, and the wells were filled with 100 μ l of DMEM containing 25 mM HEPES, pH 7.0, and 0.25% BSA. Subsequently, 50 μ l containing 5,000 to 10,000 T cells that had been labeled with [⁵¹Cr]Na₂CrO₄ (350 to 600 mCi/mg; Amersham International, Buckinghamshire, UK; 0.1 mCi/5 \times 10⁶ cells, followed by washing three times in DMEM) were added. Adhesion was allowed for 30 min at 37°C in the CO₂ incubator. Non-adherent cells were removed by washing three times with 0.3 ml of PBS-BSA. The adherent cells were solubilized for 30 min with 50 μ l of 0.25% SDS, 1% Triton X-100, 0.5% sodium deoxycholate, and 5 mM EDTA and counted in a gamma counter (Multi-Prias 1, Packard Instrument Co., Downers Grove, IL). Inhibition was calculated according to the formula

$$\% \text{ inhibition} = \left(1 - \frac{\text{cpm with mAb to test}}{\text{cpm with irrelevant control mAb}} \right) \times 100.$$

For inhibition experiments EC and lymphocytes were preincubated with mAb and used without further washing.

Fluorescence analysis. A suspension of EC was obtained by digestion of EC cultures with collagenase (125 U/ml in 137 mM NaCl, 4 mM KCl, and 10 mM HEPES, pH 7.0) for 5 min at 37°C. EC and T cells were washed in ice-cold PBS containing 0.25% BSA and 0.02% NaN₃ and suspended to 2 \times 10⁶ (EC) or 10 \times 10⁶ (T lymphocytes) cells/ml. Fifty μ l were transferred to 5-ml tubes and incubated for 30 min at 4°C with 50 μ l 1/500 diluted mAb containing ascites. The cells were washed with 2 ml of PBS containing 0.25% BSA and 0.02% NaN₃ and incubated with 50 μ l of affinity-purified goat anti-mouse IgG F(ab')₂-FITC conjugate (100 μ g/ml antibody; Zymed, San Francisco, CA). The cells were washed once in PBS containing 0.02% NaN₃ in the presence of 0.25% BSA and once in PBS containing 0.02% NaN₃ in the absence of BSA, fixed in 1.5% paraformaldehyde in PBS, and measured in the FACScan (Becton and Dickinson, Mountain View, CA). Antigen expression was presented as the mean fluorescence intensity (arbitrary units).

RESULTS

Cell Surface Expression of T Cell Clones and EC

T cells. Analysis of cell surface molecules expressed by the cloned T cells JS132, JS136, LAD1, LAD4, LAD6.6, and LAD19 is presented in Table II. No differences were observed between two cytotoxic LAD clones (LAD1 and LAD6.6) (P. Van de Wiel-van Kemenade, unpublished observations) and two non-cytotoxic LAD clones (LAD4 and LAD19). Furthermore, LFA-1⁺ and LFA-1⁻ T cell clones did not differ significantly in the expression of cell surface molecules other than leukocyte integrins. Notably, all of the T cell clones tested expressed an equal amount of VLA-4, irrespective whether they did or did

⁷ J. Leeuwenberg, Maastricht. The mAb is directed to a different epitope as ENA-1 (43).

TABLE I
Mab used for inhibition of adhesion of T cell clones to EC

Ag	mAb	Ig Sub-class	Reference	Concentration of purified IgG ($\mu\text{g/ml}$)	Dilution of ascites
gp100	NKI/beteb	IgG2b	23	25	1/100
HLA class I	W6/32	IgG2a	24	48	1/100
HLA-DR/DP	Q5/13	IgG2a	25	18	
HLA-DQ	SPV-L3	IgG1	26	41	
CD3	SPV-T3b	IgG2a	26, 27		1/100
CD4	CLB-T4/1	IgG1	28		1/100
CD8	WT82	IgG2a	29		1/100
CD28	15E8	IgG1	30		1/100
CD11a, LFA-1	NKI-L7	IgG1	31	22	1/100
CD11c, p150/95	S-HCl 3	IgG2	32		1/100
CD18, β_2 integrin	CLB-LFA-1/1	IgG1	33	45	
CD54, ICAM-1	F10.2	IgG1	^a		1/100
CD58, LFA-3	TS2/9	IgG1	34		1/100
CD2	6G4	IgG1	35		1/100
CD44, Hermes-1	NKI-P1	IgG1	36		1/100
CDw49d, VLA-4	HP2/1	IgG1	37		1/100
VLA-5	SAM-1	IgG2b	38		1/100
CDw49f, VLA-6	GOH3	IgG2a ^b	39		1/100
CD51, VNR	NKI-M9	IgG1	40	25	
VCAM-1	2G7	IgG1	41		1/250
LECAM-1	DREG-56	IgG1	42	25	
ELAM-1	ENA-2	IgG1	^c	10	

^a P. Bloemen, G. Moldenhauer, M. Van Dijk, H.-J. Schuurman, F. H. J. Gmelig Meyling, and A. C. Bloem. Multiple ICAM-1 (CD54) epitopes are involved in homotypic B cell adhesion. *Scand. J. Immunol.* 1992, in press.

^b Rat mAb; others are murine mAb.

^c See Footnote 7.

TABLE II
Phenotypic analysis of LFA-1⁻ and LFA-1⁺ T cell clones as analyzed in the FACScan

Ag	mAb	LAD1	LAD4	LAD6.6	LAD19	JS132	JS136
HLA class I	W6/32	++ ^a	++	++	++	++	++
HLA-DR/DP	Q5/13	++	++	++	++	++	++
HLA-DQ	SPV-L3	+	++	++	+	++	++
CD3	SPV-T3b	++	++	++	++	++	++
CD4	CLB-T4/1	-	++	++	++	-	++
CD8	WT82	++	-	-	-	++	-
CD28	15E8	-	-	-	-	-	-
CD11a, LFA-1	NKI-L7	-	-	-	-	++	++
CD11b, Mo-1	NKI-Bear1	-	-	-	-	+	++
CD11c, p150/95	B-ly6	-	-	-	-	(+)	(+)
CD18, β_2 integrin	CLB-LFA-1/1	-	-	-	-	++	++
CD54, ICAM-1	F10.2	+	+	+	+	+	+
CD58, LFA-3	TS2/9	+	+	+	+	+	+
CD2	CLB-T11.1/1	++	++	++	++	++	++
CD44, Hermes-1	NKI-P1	++	++	++	++	++	++
CD29, β_1 integrin	TS2/16	++	++	++	++	++	++
CDw49b, VLA-2	CLB-10G11	(+)	(+)	(+)	(+)	(+)	(+)
CDw49c, VLA-3	J143	(+)	-	(+)	-	-	-
CDw49d, VLA-4	HP1/3, HP2/1 ^b	++	++	++	++	++	++
CDw49e, VLA-5	SAM-1	+	+	+	+	+	+
CDw49f, VLA-6	GOH3	-	-	-	-	-	-
CD41, β_3 integrin	C17	-	-	-	-	-	-
CD51, VNR	NKI-M7	-	-	-	-	-	-
LECAM-1	Leu8	NT ^c	-	NT	-	NT	-
CD25, IL-2R	CLB-IL2R/1	+	++	+	+	+	+
CD64, FcR I	32.2	-	-	-	-	-	-
CD32, FcR II	IV.3	-	-	-	-	-	-
CD16, FcR III	CLB-FcR-gran1	-	-	-	-	-	-

^a Mean fluorescence intensity; -, < 10; (+), < 20; +, < 50; ++, \geq 50.

^b Similar results were obtained with each mAb.

^c NT, not tested.

not express the β_2 -chain of the leukocyte integrins (CD18). A striking feature was that in contrast to granulocytes and T lymphocytes that had been isolated from peripheral blood by centrifugal elutriation (not shown), the T cell clones lacked expression of LECAM-1, not only 1 wk after restimulation of the cells with allogeneic lymphocytes (feeder cells) and PHA but also after culturing the cells for another wk in the presence of IL-2 only.

EC. The expression of cell surface molecules on EC is shown in Figure 1. After stimulation of EC with rTNF- α VCAM-1 was expressed de novo whereas the expression of ICAM-1 (CD54) had increased markedly, and the expression of the Hermes-1 Ag (CD44) and HLA-I class I

molecules had increased slightly. No expression was noticed of the HLA class II molecules HLA-DR/DP or HLA-DQ. Expression of the rapidly inducible adhesion molecule ELAM-1, which was highly expressed after 4 h of stimulation with rTNF- α (not shown), had faded away after 24 h in the presence of the cytokine.

Comparison of adhesion of T cell clones to EC

Adhesion of LFA-1⁺/LFA-1⁻ T cells. We first investigated the ability of different T cell clones to adhere to both unstimulated and rTNF- α -stimulated EC. Maximal adhesion of both LFA-1⁺ and LFA-1⁻ T cells was obtained after stimulation of EC with rTNF- α for 24 h (Fig. 2).

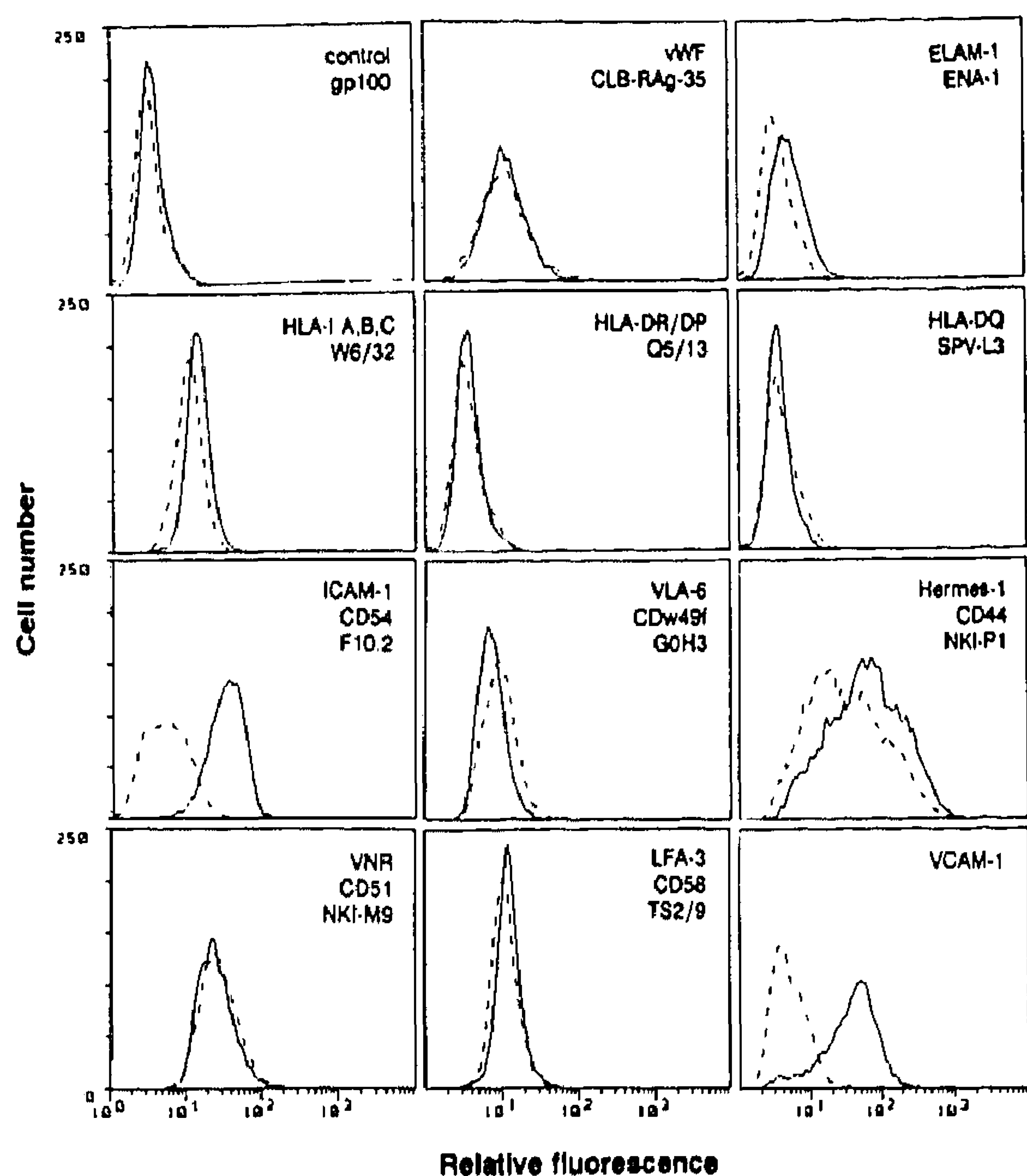


Figure 1. Phenotypic expression of surface molecules on EC as analyzed in the FACScan. EC were stimulated with 100 U/ml rTNF- α for 24 h (—; unstimulated EC, ---).

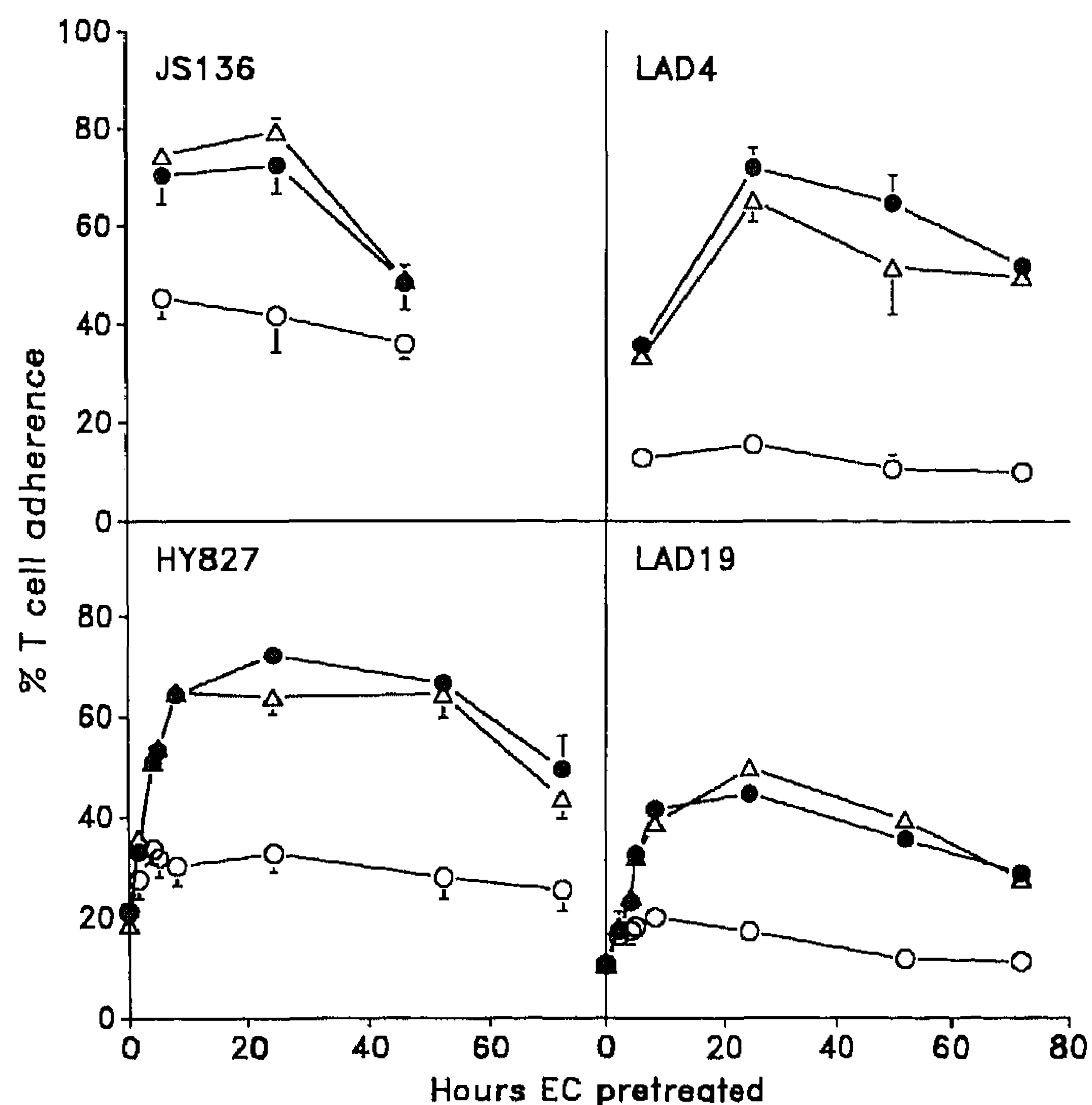


Figure 2. Adhesion of T cell clones to EC. EC were cultured in the presence of rTNF- α (●, 100 U/ml; Δ, 200 U/ml) or medium (O, control). JS136 and HY827 are antigen-specific CD4⁺ LFA-1⁺ T cell clones; LAD4 and LAD19 are CD4⁺ LFA-1⁻ T cell clones. The SE bars represent three independent tests within a representative of two experiments. To enable proper comparison, the tests with JS136 and LAD4 were performed simultaneously, as were the tests with HY827 and LAD19.

Although the LFA-1⁻ T cell clones bound to untreated EC in much fewer numbers than the LFA-1⁺ cells, the LFA-1⁻ and LFA-1⁺ T cells showed a similar enhancement of adhesion when the EC were pretreated with rTNF- α (Figs. 2 and 3). From these results we conclude that although LFA-1⁻ T cells showed reduced adhesion to EC because of absence of LFA-1/ICAM-1 and LFA-1/ICAM-2 interactions, T cells from LAD patients possess additional

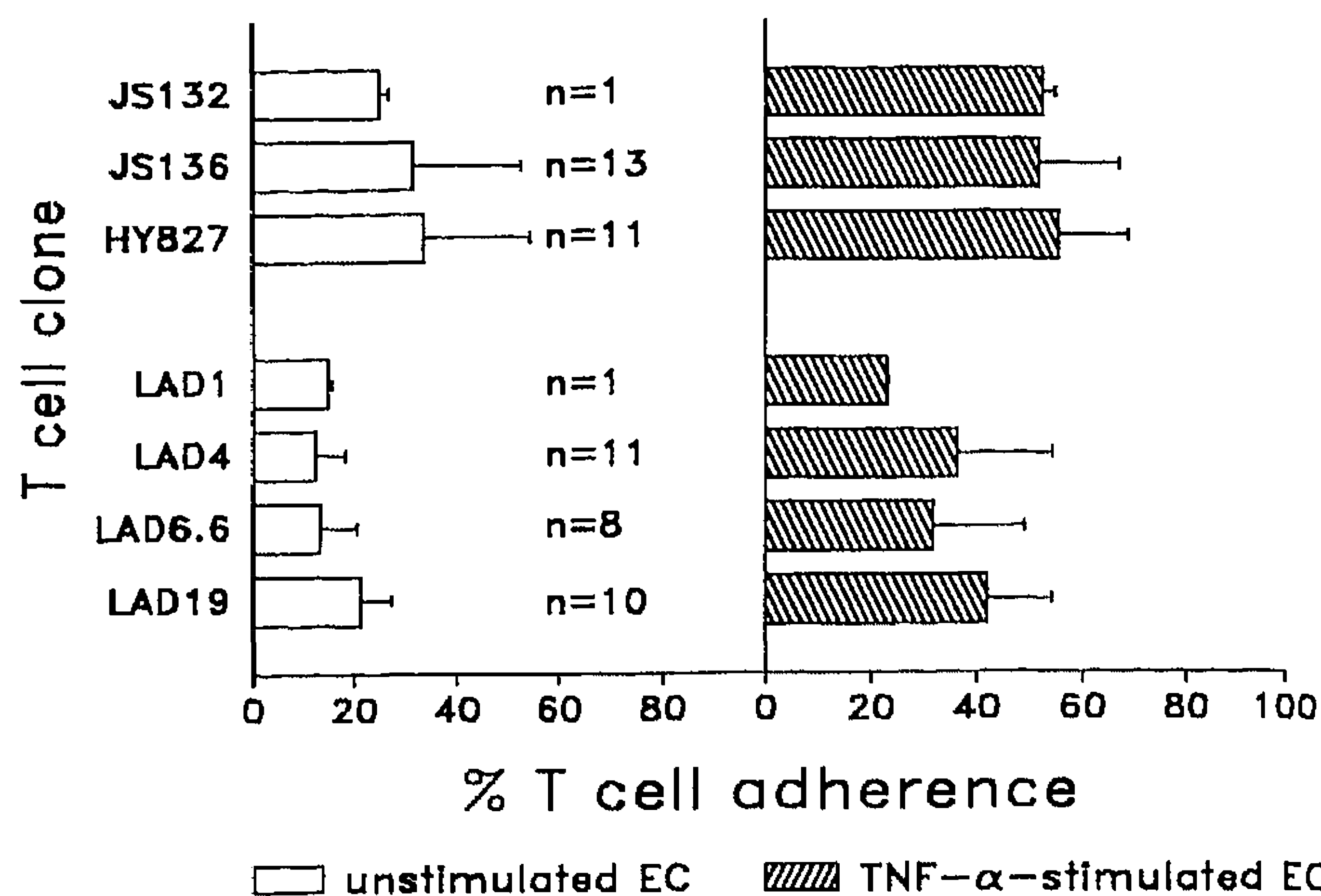


Figure 3. Adhesion of T cell clones to EC. EC were cultured in either the absence or the presence of rTNF- α (24 h, 100 U/ml). JS132 (LFA-1⁺) and LAD1 (LFA-1⁻) are CD8⁺ cytotoxic T cell clones. LAD6.6 is a CD4⁺ LFA-1⁻ cytotoxic T cell clone. See Figure 2 for a description of the other clones. The SE bars represent the number of different experiments performed (indicated by n). Three independent tests were performed within each experiment.

molecules that are involved in adhesion to cytokine-stimulated EC.

Cytotoxic/noncytotoxic T cells. No marked differences in adhesion to EC were obtained between the LFA-1⁺/CD4⁺ cytotoxic T cell clone JS136 and the tetanus toxoid-specific T helper cell clone HY827 (Fig. 2) or between the LFA-1⁻/CD4⁺ cytotoxic T cell clone LAD6.6 compared with the noncytotoxic T cell clones LAD4 and LAD19 (Fig. 3).

CD4/CD8 T cells. Adhesion to EC of the LFA-1⁺/CD4⁺ T cell clones JS136 and HY827 was similar to that of the LFA-1⁺/CD8⁺ T cell clone JS132. Likewise, adhesion of the LFA-1⁻/CD4⁺ T cell clones LAD4, LAD6.6, and LAD19 was similar to that of the LFA-1⁻/CD8⁺ T cell clone LAD1 (Fig. 3).

Structures involved in adhesion of T cell clones to EC

To investigate which molecules are responsible for T cell adhesion, we incubated T cells as well as EC with mAb before the adhesion assay. Effects on adhesion to EC of the LFA-1⁺/CD8⁺ cytotoxic T cell clone JS132 and the LFA-1⁻/CD8⁺ cytotoxic T cell clone LAD1, which, because of considerably slower growth rates than the CD4⁺ clones, were not available in sufficient amounts, were tested with a limited set of mAb (HLA class I, HLA-DR/DQ, CD3, CD8, LFA-1, p150,95, the β_2 -chain of the leukocyte integrins, the Hermes-1 Ag (CD44), and the vitronectin receptor). The inhibition of JS132 by mAb was comparable to that obtained with JS136 and HY827. Likewise, the inhibition of adhesion of LAD1 by mAb was comparable with that of LAD4, LAD6.6, and LAD19 (not shown). mAb to VCAM-1 was tested with JS136, HY827, and LAD19 (see below). No difference was noticed whether preincubation of T cells with mAb was performed at 4 or 37°C or whether preincubation of T cells and EC was performed for 30 min vs 60 min (compare Fig. 4 with Figs. 6 and 7, and Fig. 5 with Fig. 6).

LFA-1/ICAM-1. Several groups have reported that the leukocyte integrins play an important role in adhesion to EC. Notably, LFA-1 (CD11a) is involved in adhesion of unstimulated and phorbol dibutyrate-stimulated peripheral T lymphocytes to unstimulated EC (14, 15, 53, 54). In our experiments adhesion of LFA-1⁺ T cell clones to

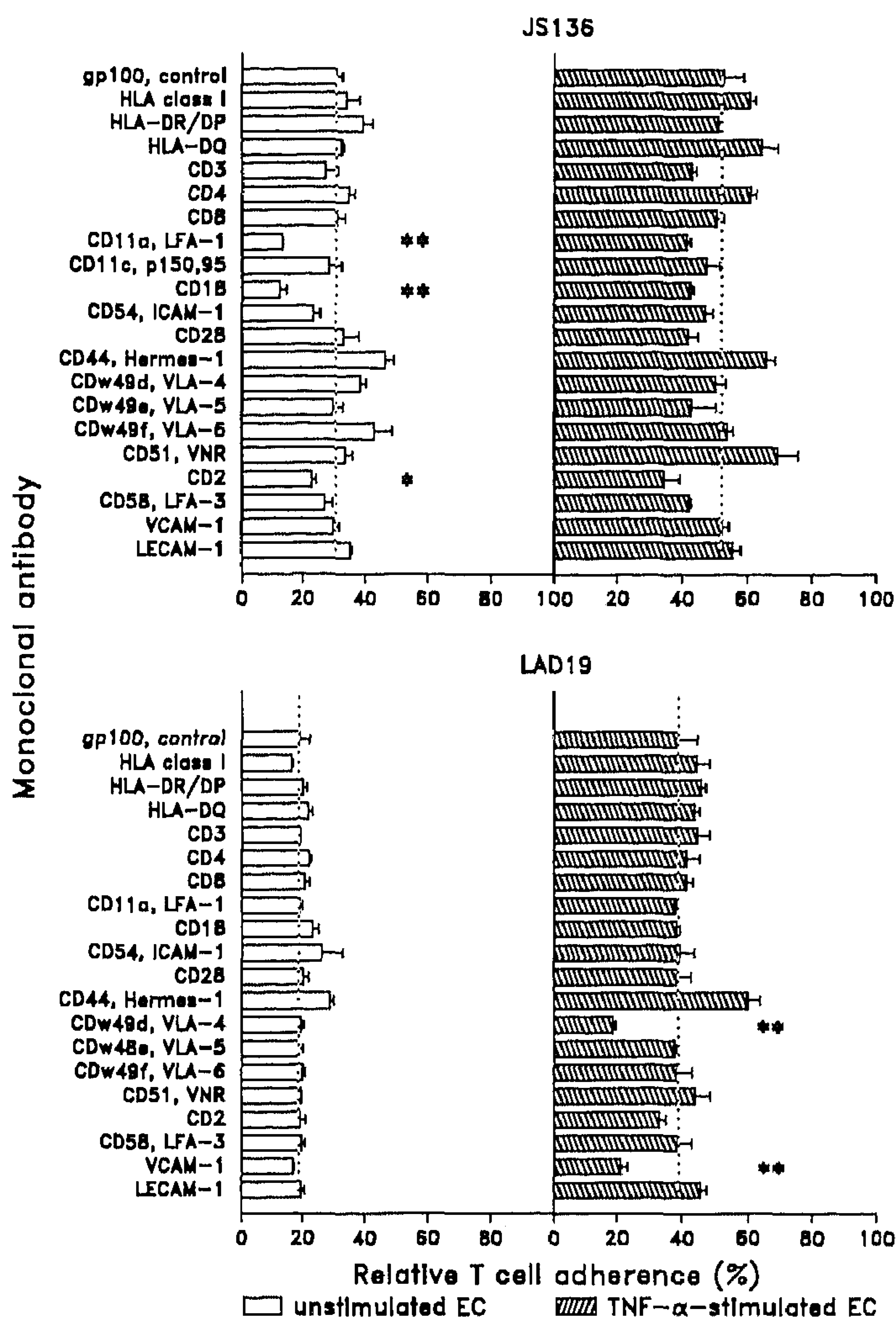


Figure 4. Inhibition of adhesion of LFA-1⁺ (JS136) and LFA-1⁻ (LAD19) T cells to EC by different mAb. Stimulation of EC was with 100 U/ml rTNF- α for 24 h. Preincubation with mAb was for 60 min at 37°C. The SE bars represent three independent tests of an average of five experiments. The asterisks represent significant reduction of adhesion in a one-sided *t*-test compared with the incubation lacking mAb, in which *, *p* = 0.01 and **, *p* = 0.005. mAb to gp100, a melanosomal protein specific for cells of the melanocyte lineage (27), was used as a control antibody.

unstimulated EC was inhibited 58% (mean of 24 experiments) by mAb to the LFA-1 α -chain (CD11a) or the common β_2 -chain of the leukocyte integrins (CD18) (see also JS136 and HY827 in Figs. 6 through 7). Inhibition of adhesion of LFA-1⁺ T cell clones by mAb to either LFA-1 (CD11a) or CD18 was either much lower or absent when the EC had been stimulated with rTNF- α (Figs. 4 through 7). Results obtained after simultaneous treatment of LFA-1⁺ T cell clones with mAb to CD11a and CD18 were similar to those obtained with treatment with either mAb alone (Fig. 5). With mAb to ICAM-1 (CD54), which is one of the counterstructures of LFA-1 on endothelial cells, inhibition of adhesion of LFA-1⁺ T cells to unstimulated EC was far less (23% inhibition of adhesion; Fig. 4).

Effect of phorbol ester. PMA has been shown to enhance LFA-1-dependent adhesion of T lymphocytes to cultured EC (14, 15, 53, 54). Anti-LFA-1 antibodies inhibited (69% inhibition) the 2.5-fold enhanced adhesion of HY827 cells that had been pretreated with the phorbol ester PMA to unstimulated EC (data not shown). Pretreatment of LFA-1⁺ T cells with PMA had no effect on the adhesion to rTNF- α -stimulated EC. Together, our results with cloned T cells show that LFA-1 is the main structure

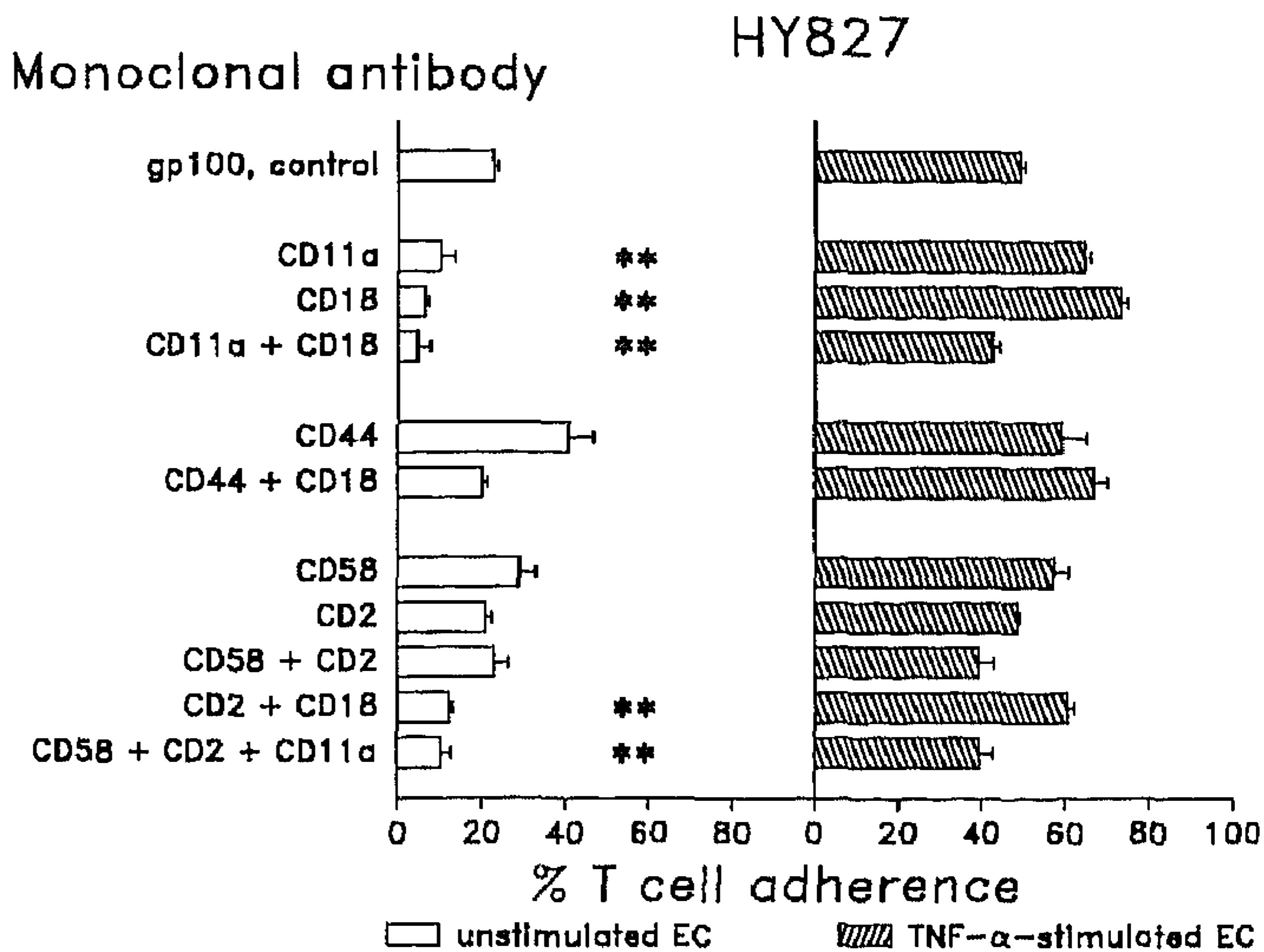


Figure 5. Adhesion of HY827 T cells to EC after simultaneous incubation with different mAb. Preincubation with mAb was for 60 min at 37°C. Stimulation of EC was for 24 h with 100 U/ml rTNF- α . Preincubation with mAb was for 60 min at 37°C. The SE bars represent three independent tests within the experiment. One representative out of three experiments is shown. See the legend of Figure 4 for an explanation of the asterisks.

involved in adhesion of T cells to unstimulated but not to rTNF- α -stimulated EC (24 h) and confirm the finding of others (14, 15, 53, 54) that PMA primarily stimulates LFA-1-dependent adhesion.

Role of VLA-4/VCAM-1. A structure on mouse lymphocytes showing homology with the human VLA-4 molecule is involved in adhesion to Peyer's patches and mucosal HEV (55). Several investigators have reported that in man adhesion of T lymphocytes and some T and B lymphoid cell lines to TNF- α - or IL-1-stimulated EC can be inhibited by antibodies both against VLA-4 and its counterstructure on the EC VCAM-1 (6, 41, 54, 56–58). However, in our experiments the binding of LFA-1⁺/CD4⁺ cytotoxic and T helper cell clones JS136 and HY827 to EC was not affected either by anti-VLA-4 or by anti-VCAM-1 antibody, whether or not these EC had been stimulated with rTNF- α (Figs. 4, 6, and 7). Neither did incubation with anti-LFA-1 in the presence of anti-VLA-4 antibodies reduce the adhesion of these LFA-1⁺ T cells to TNF- α -stimulated EC (Fig. 6). This was not because of the anti-VLA-4 antibody used inasmuch as it was effective in inhibiting (70%) adhesion of the LFA-1-positive/ICAM-1-negative T cell line Jurkat to EC that were stimulated by rTNF- α . Adhesion to rTNF- α -stimulated, but not unstimulated, EC of the cytotoxic (LAD6.6) and the two noncytotoxic (LAD4 and LAD19) CD4⁺ LFA-1⁻ T cell clones tested was inhibited (average inhibition of 56%; mean of 13 experiments) by anti-VLA-4 (Figs. 6 and 7). An inhibition of 33% was obtained with mAb to VCAM-1 (Fig. 4). These results indicate that LFA-1⁻ T cells, like the LFA-1⁺ cell line Jurkat, adhere to rTNF- α -stimulated, but not to unstimulated, EC via the receptor/ligand pair VLA-4/VCAM-1. Since the adhesion of Jurkat cells to EC resembled more that of LFA-1⁻ than that of LFA-1⁺ T cells, the LFA-1 molecules on Jurkat cells are possibly not, or not fully, functional.

CD44. The Hermes-1 Ag is mainly involved in binding of PBL to mucosal and synovial lymph node and to a lesser extent to peripheral lymph node HEV (59). Previously, a different mAb (NKI-P1) to this Ag was prepared which also inhibited adhesion of PBL to peripheral lymph

Monoclonal antibody

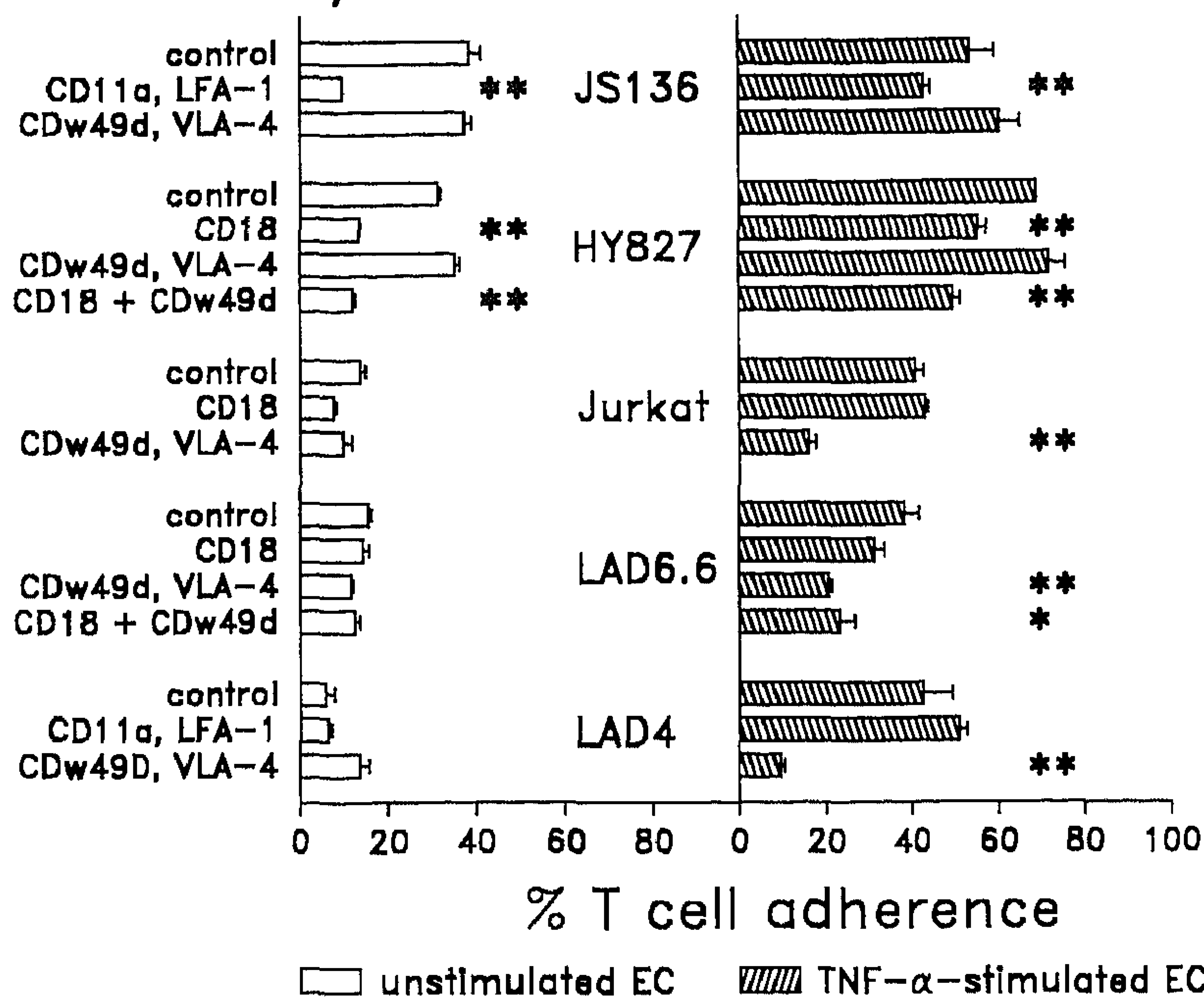


Figure 6. Inhibition of adhesion of Jurkat, JS136, HY827, LAD4, and LAD6.6 to EC by mAb directed against VLA-4 (CDw49d), LFA-1 (CD11a), the β_2 -chain of the leukocyte integrins (CD18), or a mixture of these mAb. Stimulation of EC was for 24 h with 100 U/ml rTNF- α . Preincubation of T cells with mAb was for 30 min at 4°C. The SE bars represent three independent tests within the experiment. A representative of two experiments is shown. See the legend of Figure 4 for an explanation of the asterisks.

Monoclonal antibody

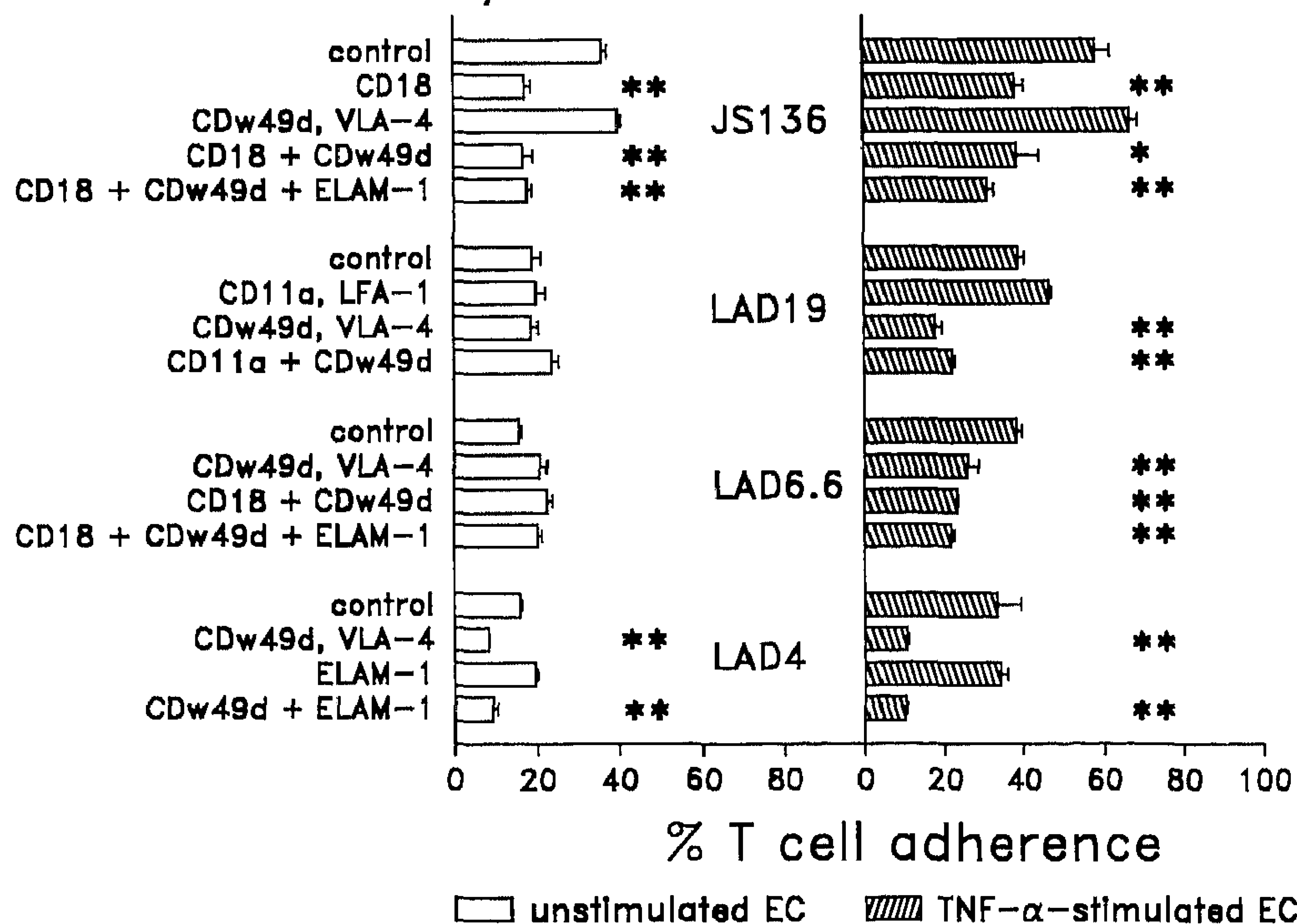


Figure 7. Inhibition of adhesion of JS136, LAD6.6, and LAD4 to EC by mAb directed against VLA-4 (CDw49d), LFA-1 (CD11a), the β_2 -chain of the leukocyte integrins (CD18), ELAM-1, or a mixture of these mAb. Stimulation of EC was for 24 h with 100 U/ml rTNF- α . Preincubation of T cells with mAb was for 30 min at 4°C, and preincubation of EC with mAb to ELAM-1 was for 30 min at 37°C. The SE bars represent three independent tests within the experiment. A representative of two experiments is shown. See the legend of Figure 4 for an explanation of the asterisks.

node HEV but at the same time stimulated homotypic aggregation of cells of the EBV-transformed cell line JY (36). In tests with cloned T cells, however, we never observed inhibition of adhesion to (TNF- α -stimulated) EC. Instead, in 6 out of 14 experiments we observed more than 50% increased adhesion of cloned T cells by this mAb to unstimulated EC (Figs. 4 and 5). The increased adhesion was observed with both cytotoxic and noncytotoxic LFA-1⁺ and LFA-1⁻ CD4⁺ T cell clones and was

not dependent on preincubation of the T cells with anti-CD44 at 37°C compared with 4°C. The enhanced adhesion to unstimulated EC was only partially reduced by mAb to CD18 (Fig. 5). There are several explanations for this phenomenon, which will be discussed below.

No marked inhibitory or stimulatory effects on adhesion of the LFA-1⁺ or LFA-1⁻ T cell clones to rTNF- α -stimulated or unstimulated EC were observed with any of the other mAb tested, neither with mAb to CD8 and

CD4, nor with mAb to LFA-3 and CD2 (Figs. 4 and 5). LFA-3 and CD2 have been shown recently to be involved in EC-augmented IL-2 production in T cells (60). In analogy with the observation with neutrophils (8, 42, 61), the cultured T cell clones probably lose LECAM-1 expression because they are activated during *in vitro* culturing. In accordance with this finding, no inhibition of adhesion of LFA-1⁺ or LFA-1⁻ T cells to EC was observed with the inhibiting mAb DREG-56 (42) (Fig. 4). ELAM-1, which is implicated in preferential adhesion of memory T lymphocytes to EC in the skin (11, 62), was, as discussed before, also not present any more on the EC after incubation with rTNF- α for 24 h (Fig. 1), and we found no inhibition of adhesion of cloned T cells after incubation of stimulated EC with anti-ELAM-1 mAb (Fig. 7).

DISCUSSION

The main points emerging from this study with T cell clones are the following. 1) LFA-1⁻ T cells show reduced adhesion to EC compared with LFA-1⁺ cells. 2) Stimulation of EC with TNF- α enhances adhesion of LFA-1⁺ and LFA-1⁻ T cells. 3) Adhesion of LFA-1⁺ T cells to unstimulated EC is LFA-1 dependent. 4) Adhesion of PMA-activated T cells to unstimulated EC is increased and is LFA-1 dependent. 5) VLA-4 mediates adhesion of LFA-1⁻ T cells to TNF- α -stimulated EC but not unstimulated EC. 6) Because VLA-4 does not mediate adhesion of cloned LFA-1⁺ T cells to TNF- α -stimulated (24 h) EC and because LECAM-1 or ELAM-1 did not contribute to adhesion of these cells, a new, as yet undefined, adhesion structure(s) must exist.

LECAM-1, which is implicated in adhesion of (memory) T cells and neutrophils to peripheral lymph node HEV (8, 9, 42), was not expressed by the T cell clones studied. Preliminary experiments (not shown) show that upon activation with PMA, peripheral T cells lose LECAM-1 expression similarly to what has been described for neutrophils (61). Therefore, LECAM-1 expression is not implicated in T cell adhesion to EC in our study.

LFA-1/ICAM-1 and VLA-4/VCAM-1 interactions have been reported to play a role in adhesion of peripheral blood T lymphocytes to cultured EC (4, 14, 41, 53, 58). In addition, ELAM-1 has been shown recently to mediate adhesion of CD4⁺ memory cells to 4- to 6-h rIL-1-stimulated EC (41, 54). We have deliberately stimulated the EC for 24 h with rTNF- α because after this period of time maximal adhesion of cloned T cells was obtained (Fig. 2). Preliminary experiments indicate also that after 24 h of stimulation of EC with rIL-1 β maximal adhesion of these T cells is obtained (data not shown). However, after this prolonged incubation ELAM-1 is hardly expressed on EC (10, 43), thus excluding a role of ELAM-1 in this study.

VCAM-1 and ICAM-1, the Hermes-1 Ag (CD44), were up-regulated after stimulation of EC with rTNF- α . Our results with T cell clones show that VLA-4/VCAM-1 is an important alternative pathway if LFA-1/ICAM-1 interactions are absent. The data with LFA-1⁺ T cell clones are in contrast with data obtained with resting or PMA-stimulated peripheral T lymphocytes, their adhesion to activated EC being inhibited by a mixture of mAb to LFA-1 and either VLA-4 or VCAM-1 (7, 41, 54). Therefore, we conclude that cloned T cells, which are cultured in the presence of IL-2 and are strongly activated (63), behave differently. Our data with LFA-1⁻ T cell clones confirm and extend for the greater part those obtained by Haskard

et al. (64) and Schwartz et al. (57), who studied adhesion of LFA-1⁻ EBV-transformed B lymphoblastoid cell lines to cultured EC. However, in contrast to the finding by Schwartz et al. (57) we generally observed that adhesion of LFA-1⁻ T cell clones to unstimulated EC could not be inhibited by an antibody to VLA-4 α . In our hands unstimulated EC did not express the counterstructure VCAM-1. Possibly differences in culture conditions of EC, notably the presence of xenogeneic serum and ECGF shortly before the adhesion experiment and the presence of serum during the experiments described by Schwartz et al., may have contributed to slight stimulation of EC. We avoided the use of serum during the adhesion test, and 2 days before the assay the EC were deprived of ECGF and heparin. Moreover, we cultured the EC in human serum only. However, in one experiment with LAD4 cells (Fig. 7), in which a particular batch of human serum was used, the EC showed morphologically in culture an elongated appearance similar to TNF- α -activated EC. In this particular case inhibition by anti-VLA-4 can be observed, supporting the notion that particular culture conditions may stimulate VCAM-1 expression.

Recently, Kavanaugh et al. (58) also demonstrated that LAD T cells bind to endothelium by a VLA-4/VCAM-1 dependent mechanism (58). In contrast with their findings, we did not observe VLA-4-mediated binding of LAD T cells to unstimulated EC, but this may be a result of differences in the culture conditions, as discussed above. Furthermore, we observed repeatedly that adhesion of LFA-1⁺ T cells to EC (except Jurkat, which is discussed below) and to TNF- α -stimulated EC could not significantly be inhibited by VLA-4 or VCAM-1 antibodies even in the presence of anti-LFA-1 or anti-CD18 antibodies. This discrepancy may be a result of differences in the T cell clones used or differences in stimulation of EC (4 vs 24 h). Binding of LFA-1⁻ T cell clones to stimulated EC is dependent on VLA-4 both in our studies and in those of Kavanaugh.

A low number of Jurkat T cells, which showed a normal expression of LFA-1, adhered to unstimulated EC. In contrast to LFA-1⁺ T cell clones, the enhanced adhesion of Jurkat T cells to stimulated EC could be inhibited by mAb to VLA-4, similar to what we observed with LFA-1-deficient T cells. These and earlier observations that LFA-1 needs to be activated to enable cell adhesion (65, 66) indicate that the LFA-1 molecules on Jurkat cells might not be activated. Preliminary experiments with mAb NKI-L16 directed against the α -chain of LFA-1, which is able to activate LFA-1 (67), confirm this observation (Y. van Kooyk, unpublished observations).

Stimulation of LFA-1⁺ T cells by PMA increased adhesion of these cells to unstimulated EC, and this effect was completely LFA-1 dependent. This is in concordance with previous observations (66) and those of others (65), suggesting that LFA-1-dependent adhesion requires activation of LFA-1. In contrast, we repeatedly found that PMA was not able to enhance adhesion of LFA-1⁺ T cells to TNF- α -stimulated EC despite a much higher ICAM-1 expression on the EC. Similar results have been obtained by Kavanaugh et al. (58) with LFA-1⁺ T cell clones. This is in contrast to the data obtained with LFA-1⁺ T lymphocytes from peripheral blood, which, upon activation with phorbol ester, showed enhanced adhesion to IL-1-stimulated EC (14, 53, 54). This enhanced adhesion of phorbol ester-activated LFA-1⁺ T lymphocytes to IL-1-

stimulated EC was, however, inhibited by mAb to LFA-1 to the level obtained with resting T lymphocytes (14, 53). These results suggest that phorbol ester-enhanced adhesion of resting LFA-1⁺ lymphocytes to IL-1-activated EC is also LFA-1 dependent. However, our findings suggest that compared with resting T lymphocytes from peripheral blood, LFA-1⁺ T cell clones use a different pathway for adhesion to activated EC.

We cannot completely rule out that the VLA-4/VCAM-1 pathway does not play a role in adhesion of the LFA-1⁺ T cell clones to EC. The possibility exists that an as yet unknown receptor/ligand pair contributes that much to adhesion of cloned T cells to EC that VLA-4/VCAM-1 interaction is obscured. However, other explanations are also possible, such as signals generated after the interaction of LFA-1 with ICAM-1 and/or ICAM-2, which may stimulate other adhesion pathways. A role for CD11b (which is highly expressed on JS136 cells) in adhesion to activated EC can, however, be excluded since mAb to the β_2 -subunit of the leukocyte integrins did not inhibit the adhesion of JS136 or HY827 to TNF- α -stimulated EC. An additional possibility to be considered is that the LFA-1/VLA-4-independent component of binding represents adhesion to matrix components that have been secreted upon activation of the EC rather than to an EC ligand. More experiments are necessary to prove this.

In a number of experiments the adhesion of LFA-1⁺ and LFA-1⁻ T cells to unstimulated and rTNF- α -stimulated EC was markedly influenced by mAb to the Hermes-1 Ag (CD44), known to induce homotypic aggregation of the EBV-transformed B cell line JY within 35 min (36). This result may be explained in several ways. Preincubation of T cells with anti-CD44 may have caused T cell aggregation, resulting in a higher number of T cells that bound to EC because of T cell/T cell interactions. However, microscopic examination at the end of the test did not support this possibility. Alternatively, cross-linking by mAb of CD44 molecules on EC and lymphocytes may explain enhanced binding. Finally, analogous to the observations with mAb NKI-L16 to LFA-1 (67), enhanced affinity of the CD44 molecule for its ligand hyaluronate (68) after binding of the antibody to a particular epitope of the CD44 antigen cannot be excluded.

Because adhesion of LFA-1-expressing T cell clones to TNF- α -stimulated EC could not be inhibited significantly by any of the mAb examined, we speculate that the latter adhesion is dependent on a hitherto unknown adhesion molecule.

REFERENCES

1. Yednock, T. A., and S. D. Rosen. 1989. Lymphocyte homing. *Adv. Immunol.* 44:313.
2. Pohlman, T. H., K. A. Stanness, P. G. Beatty, H. D. Ochs, and J. M. Harlan. 1986. An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1, and tumor necrosis factor- α increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol.* 136:4548.
3. Cavender, D., Y. Saegusa, and M. Ziff. 1987. Stimulation of endothelial cell binding of lymphocytes by tumor necrosis factor. *J. Immunol.* 139:1855.
4. Dustin, M. L., and T. A. Springer. 1988. Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. *J. Cell Biol.* 107:321.
5. Staunton, D. E., M. L. Dustin, and T. A. Springer. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 339:61.
6. Osborn, L., C. Hession, R. Tizard, C. Vassallo, S. Luhowskyj, G. Chi-Rosso, and R. Lobb. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 59:1203.
7. Rice, G. E., J. M. Munro, and M. P. Bevilacqua. 1990. Inducible cell adhesion molecule-110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion mechanism. *J. Exp. Med.* 171:1369.
8. Hallmann, R., M. A. Jutila, C. W. Smith, D. C. Anderson, T. K. Kishimoto, and E. C. Butcher. 1991. The peripheral lymph node homing receptor, LECAM-1, is involved in CD18-independent adhesion of human neutrophils to endothelium. *Biochem. Biophys. Res. Commun.* 174:236.
9. Spertini, O., G. S. Kansas, J. M. Munro, J. D. Griffin, and T. F. Tedder. 1991. Regulation of leukocyte migration by activation of the leukocyte adhesion molecule-1 (LAM-1) selectin. 1991. *Nature* 349:691.
10. Lusinskas, F. W., A. F. Brock, M. A. Arnaout, and M. A. Gimbrone. 1989. Endothelial leukocyte adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. *J. Immunol.* 142:2257.
11. Shimizu, Y., S. Shaw, N. Graber, T. V. Gopal, K. J. Horgan, G. A. Van Seventer, and W. Newman. 1991. Activation-independent binding of human memory T cells to adhesion molecule ELAM-1. *Nature* 349:799.
12. Geng, J.-G., M. P. Bevilacqua, K. L. Moore, T. M. McIntyre, S. M. Prescott, J. M. Kim, G. A. Bliss, G. A. Zimmerman, and R. P. McEver. 1990. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* 343:757.
13. Brandley, B. K., S. J. Swiedler, and P. W. Robbins. 1990. Carbohydrate ligands of the LEC cell adhesion molecules. *Cell* 63:861.
14. Haskard D., D. Cavender, P., Beatty, T. Springer, and M. Ziff. 1986. T lymphocyte adhesion to endothelial cells: Mechanisms demonstrated by anti-LFA-1 monoclonal antibodies. *J. Immunol.* 137:2901.
15. Prieto, J., P. G. Beatty, E. A. Clark, and M. Patarroyo. 1988. Molecules mediating adhesion of T and B cells, monocytes and granulocytes to vascular endothelial cells. *Immunology* 63:631.
16. Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalsteig, and D. C. Anderson. 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J. Exp. Med.* 160:1901.
17. Arnaout, M. A. 1990. Leukocyte adhesion molecule deficiency: Its structural basis, pathophysiology and implications for modulating the inflammatory response. *Immunol. Rev.* 114:145.
18. Jaffe, E. A., R. L. Nachman, C. G. Becker, and C. R. Minick. 1973. Culture of human endothelial cells derived from umbilical veins: Identification by morphologic and immunologic criteria. *J. Clin. Invest.* 52:2745.
19. Yssel, H., J. E. De Vries, M. Koken, W. Van Blitterswijk, and H. Spits. 1984. Serum-free medium for generation and propagation of functional human cytotoxic and helper T cell clones. *J. Immunol. Methods* 72:219.
20. Stel, H. V., K. S. Sakariassen, B. J. Scholte, E. C. I. Veerman, T. H. Van der Kwast, P. G. De Groot, J. J. Sixma, and J. A. van Mourik. 1984. Characterization of 25 monoclonal antibodies to factor VIII-von Willebrand factor: Relationship between ristocetin-induced platelet aggregation and platelet adherence to subendothelium. *Blood* 63:1408.
21. Borst, J., H. Spits, A. Voordouw, E. de Vries, A. Boylston, and J. E. de Vries. 1986. A family of T-cell receptor molecules expressed on T-cell clones with different specificities for allomajor histocompatibility antigens. *Hum. Immunol.* 17:426.
22. Yssel, H., D. Blanchard, A. Boylston, J. E. De Vries, and H. Spits. 1986. T cell clones which share T cell receptor epitopes differ in phenotype, function and specificity. *Eur. J. Immunol.* 16:1187.
23. Vennegoor, C., P. Hageman, H. van Nohuijs, D. J. Ruiter, J. Calafat, P. J. Ringens, and P. Rümke. 1988. A monoclonal antibody specific for cells of the melanocyte lineage. *Am. J. Pathol.* 130:179.
24. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens: New tools for genetic analysis. *Cell* 14:9.
25. Quaranta, V., L. E. Walker, M. A. Pellegrino, and S. Ferrone. 1980. Purification and immunologically functional subsets of human Ia-like antigens on a monoclonal antibody (Q5/13) immunoadsorbent. *J. Immunol.* 125:1421.
26. Spits, H., G. Keizer, J. Borst, C. Terhorst, A. Hekman, and J. E. De Vries. 1984. Characterization of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. *Hybridoma* 2:423.
27. Spits, H., J. Borst, M. Giphart, J. Coligan, C. Terhorst, and J. E. De Vries. 1984. HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. *Eur. J. Immunol.* 14:229.
28. Rotteveel, F. T. M., E. Braakman, B. Robbe, and C. J. Lucas. 1988. Recognition of influenza virus-infected B-cell lines by human influenza-specific CTL. *Cell. Immunol.* 111:473.
29. Tax, W. J. M., H. F. M. Leeuwenberg, H. W. Willems, P. J. A. Capel, and R. A. P. Koene. 1984. Monoclonal antibodies with OKT3 antigens or OKT8 antigen. In *Leukocyte Typing*, I. A. Bernard, L. Baumell, F. Dausset, C. Milstein, and S. F. Schlossman, eds. Springer Verlag, Berlin, p. 721.

30. Van Lier, R., M. Brouwer, R. De Jong, M. Groot, E. De Groot, and L. Aarden. 1989. Functional properties of the human T-cell differentiation antigen CD28. In *Leukocyte Typing*. IV. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. K. Von Dem Borne, eds. Oxford University Press, New York, p. 353.
31. Keizer, G. D., J. Borst, C. G. Figdor, H. Spits, F. Miedema, C. Terhorst, and J. E. De Vries. 1985. Biochemical and functional characteristics of the human leukocyte membrane antigen family LFA-1, Mo-1 and p150,95. *Eur. J. Immunol.* 15:1142.
32. Schwarting, R., H. Stein, and C. Y. Wang. 1985. The monoclonal antibodies α -S-HCL 1 (α -Leu-14) and α S-HCL 3 (α Leu-M5) allow the diagnosis of hairy cell leukemia. *Blood* 65:974.
33. Miedema, F., P. A. T. Tetteroo, W. G. Hesselink, G. Werner, H. Spits, and C. J. M. Melief. 1984. Both Fc receptors and lymphocyte-function-associated antigen on human T lymphocytes are required for antibody-dependent cellular cytotoxicity (killer cell activity). *Eur. J. Immunol.* 14:518.
34. Sanchez-Madrid F., A. M. Krensky, C. F. Ware, E. Robbins, J. L. Strominger, S. J. Burakoff, and T. A. Springer. 1982. Three distinct antigens associated with human T-lymphocyte-mediated cytotoxicity: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. USA* 79:7489.
35. Van Lier, R. A. W., M. Brouwer, and L. A. Aarden. 1988. Signals involved in T cell activation: T cell proliferation induced through the synergistic action of anti-CD28 and anti-CD2 monoclonal antibodies. *Eur. J. Immunol.* 18:167.
36. Pals, S. T., F. Hogervorst, G. D. Keizer, T. Thepen, E. Horst, and C. C. Figdor. 1989. Identification of a widely distributed 90-kDa glycoprotein that is homologous to the Hermes-1 human lymphocyte homing receptor. *J. Immunol.* 143:851.
37. Campanero M. R., R. Pulido, M. A. Ursa, M. Rodríguez-Moya, M. O. De Landázuri, and F. Sánchez-Madrid. 1990. An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1-independent, triggered through the human VLA-4 integrin. *J. Cell Biol.* 110:2157.
38. Keizer, G. D., A. A. Te Velde, R. Schwarting, C. G. Figdor, and J. E. De Vries. 1987. Role of p150,95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes. *Eur. J. Immunol.* 17:1317.
39. Sonnenberg, A., P. W. Modderman, and F. Hogervorst. 1988. Laminin receptor on platelets is the integrin VLA-6. *Nature* 336:487.
40. Von dem Borne, A. E. G. K., P. W. Modderman, L. G. Admiraal, and H. K. Nieuwenhuis. 1989. Platelet antibodies, the overall results. In *Leukocyte Typing*. IV. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. K. Von Dem Borne, eds. Oxford University Press, New York, p. 951.
41. Graber, N., T. V. Gopal, D. Wilson, L. D. Beall, T. Polte, and W. Newman. 1990. T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leukocyte adhesion molecule-1. *J. Immunol.* 145:819.
42. Kishimoto, T. K., M. A. Jutila, and E. C. Butcher. 1990. Identification of a human peripheral lymph node homing receptor: A rapidly down-regulated adhesion molecule. *Proc. Natl. Acad. Sci. USA* 81:2244.
43. Leeuwenberg, J. F. M., T. M. A. A. Jeunhomme, and W. A. Buurman. 1989. Induction of an activation antigen on human endothelial cells in vitro. *Eur. J. Immunol.* 19:715.
44. Giltay J. C., H.-J. M. Brinkman, P. W. Modderman, A. E. G. K. Von dem Borne, and J. van Mourik. 1989. Human vascular endothelial cells express a membrane protein complex immunochemically indistinguishable from the platelet VLA-2 (glycoprotein Ia-IIa) complex. *Blood* 73:1235.
45. Tetteroo P., P. Lansdorp, O. Leeksa, and A. von dem Borne. 1983. Monoclonal antibodies against human platelet glycoprotein IIIa. *Br. J. Haematol.* 55:509.
46. Van Lier, R. A. W., J. H. A. Boot, A. J. Verhoeven, E. R. De Groot, M. Brouwer, and L. A. Aarden. 1987. Functional studies with anti-heavy chain isotype switch-variant monoclonal antibodies: Accessory cell-independent induction of interleukin 2 responsiveness in T cells by ϵ -anti-CD3. *J. Immunol.* 139:2873.
47. Huizinga, T. W. J., C. E. Van Der Schoot, C. Jost, R. Klaassen, M. Kleijer, A. E. G. K. Von dem Borne, D. Roos, and P. A. T. Tetteroo. 1988. The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature* 333:667.
48. Fradet Y., C. Cordon-Cardo, T. Thomson, M. E. Daly, W. F. Whitmore, K. O. Lloyd, M. R. Melamed, and L. J. Old. 1984. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* 81:224.
49. Visser, L., A. Shaw, J. Slupsky, H. Vos, and S. Poppema. 1989. Monoclonal antibodies reactive with hairy cell leukemia. *Blood* 74:320.
50. Hemler, M. E., F. Sanchez-Madrid, T. J. Flotte, A. M. Krensky, S. J. Burakoff, A. K. Bhan, T. A. Springer, and J. L. Strominger. 1984. Glycoproteins of 210,000 and 130,000 m.w. on activated T cells: Cell distribution and antigenic relation to components on resting cell lines. *J. Immunol.* 132:3011.
51. Hemler, M. E., C. Huang, Y. Takada, L. Schwarz, J. L. Strominger, and M. L. Clabby. 1987. Characterization of the cell surface heterodimer VLA-4 and related peptides. *J. Biol. Chem.* 262:11478.
52. Anderson, C. L., P. M. Guyre, J. C. Whitin, D. H. Ryan, R. J. Looney, and M. W. Fanger. 1986. Monoclonal antibodies to Fc receptors for IgG on human mononuclear phagocytes: Antibody characterization and induction of superoxide production in a monocyte cell line. *J. Biol. Chem.* 261:12856.
53. Oppenheimer-Marks, N., L. S. Davis, and P. E. Lipsky. 1990. Human T lymphocyte adhesion to endothelial cells and transendothelial migration: Alteration of receptor use relates to the activation status of both the T cell and the endothelial cell. *J. Immunol.* 145:140.
54. Shimizu, Y., W. Newman, T. V. Gopal, K. J. Horgan, N. Graber, L. D. Beall, G. A. Van Seventer, and S. Shaw. 1991. Four molecular pathways of T cell adhesion to endothelial cells: Roles of LFA-1, VCAM-1, and ELAM-1, and changes in pathway hierarchy under different activation conditions. *J. Cell Biol.* 113:1203.
55. Holzmann, B., B. W. McIntyre, and I. L. Weissman. 1989. Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an α chain homologous to human VLA-4 α . *Cell* 56:37.
56. Elices, M. J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M. E. Hemler, and R. R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 60:577.
57. Schwartz B. R., E. A. Wayner, T. M. Carlos, H. D. Ochs, and J. M. Harlan. 1990. Identification of surface proteins mediating adherence of CD11/CD18-deficient lymphoblastoid cells to cultured human endothelium. *J. Clin. Invest.* 85:2019.
58. Kavanaugh, A. F., E. Lightfoot, P. E. Lipsky, and N. Oppenheimer-Marks. 1991. Role of CD11/CD18 in adhesion and transendothelial migration of T cells. *J. Immunol.* 146:4149.
59. Jalkanen, S., R. F. Bargatze, J. De Los Toyos, and E. C. Butcher. 1987. Lymphocyte recognition of high endothelium: Antibodies to distinct epitopes of an 85-95-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. *J. Cell Biol.* 105:983.
60. Hughes, C. C. W., C. O. S. Savage, and J. S. Pober. 1990. Endothelial cells augment T cell interleukin 2 production by a contact-dependent mechanism involving CD2/LFA-3 interaction. *J. Exp. Med.* 171:1453.
61. Berg, M., and S. P. James. 1990. Human neutrophils release the Leu-8 lymph node homing receptor during cell activation. *Blood* 76:2381.
62. Picker, L. J., T. K. Kishimoto, C. W. Smith, R. A. Warnock, and E. C. Butcher. 1991. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 349:796.
63. Van Kooyk, Y., P. Weder, F. Hogervorst, A. J. Verhoeven, G. Van Seventer, A. A. Te Velde, J. Borst, G. D. Keizer, and C. G. Figdor. Activation of LFA-1 through a Ca⁺⁺-dependent epitope stimulates lymphocyte adhesion. *J. Cell Biol.* 112:345.
64. Haskard, D. O., S. Strobel, M. Thornhill, C. Pitzalis, and R. J. Levinsky. 1989. Mechanisms of lymphocyte adhesion to endothelial cells: Studies using an LFA-1-deficient cell line. *Immunology* 66:111.
65. Dustin, M. L., and T. A. Springer. 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 341:619.
66. Van Kooyk, Y., P. Van de Wiel-Van Kemenade, P. Weder, T. W. Kuijpers, and C. G. Figdor. 1989. Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature* 342:811.
67. Keizer, G. D., W. Visser, M. Vliem, and C. G. Figdor. 1988. A monoclonal antibody (NKI-L16) directed against a unique epitope on the α -chain of human leukocyte function-associated antigen 1 induces homotypic cell-cell interactions. *J. Immunol.* 140:1393.
68. Miyake, K., C. B. Underhill, J. Lesley, and P. W. Kincade. 1990. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J. Exp. Med.* 172:69.